université PARIS-SACLAY | FACULTÉ DE PHARMACIE

M1 Development of drug and health products

OTU 06 : Basic structural elucidation

Practical work in Mass Spectrometry

Julia Kaffy 10th, 14th february , 7th march 2025 (6h) 2024-2025

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Mass spectrometry (MS)

- Method based on the interaction of matter with **charged particles**

- Important source of information :

    - molecular weight

    - molecular Formula (High Resolution SM)

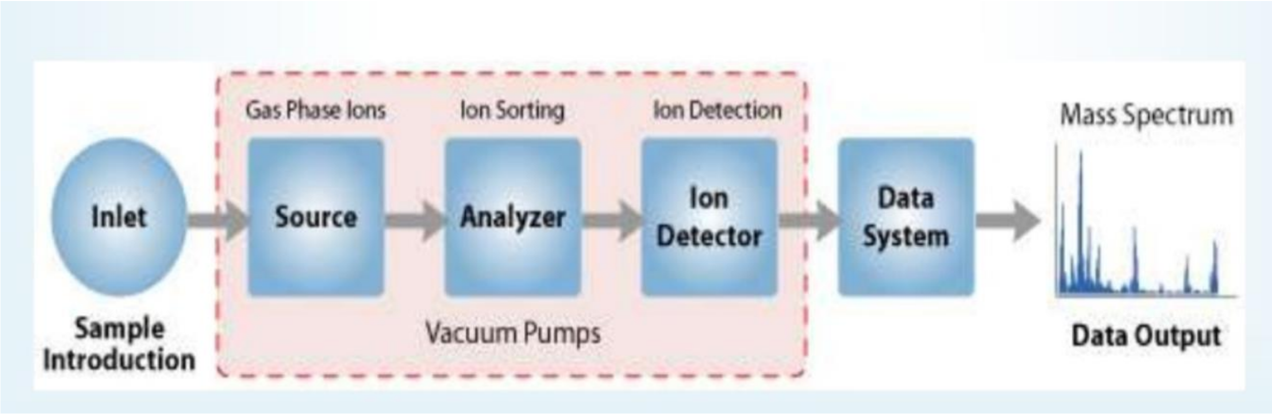
    - knowledge of **characteristic fragments** of the skeleton or of **functional groups** present in a molecule

- Method valid for small organic molecules but also macromolecules

- The MS does not give any information on the molecules but on the **molecular ions** and **the fragments** which arise from them

→ only **charged particles** are detected

Mass spectrometer



1. A small sample is ionized, usually to cations by loss of an electron. **The Ion Source**

   (Electronic Impact or EI, Chemical Ionization or CI, Electrospray Ionisation or ESI, Matrix Assited Laser Desorption-Ionisation or MALDI…)

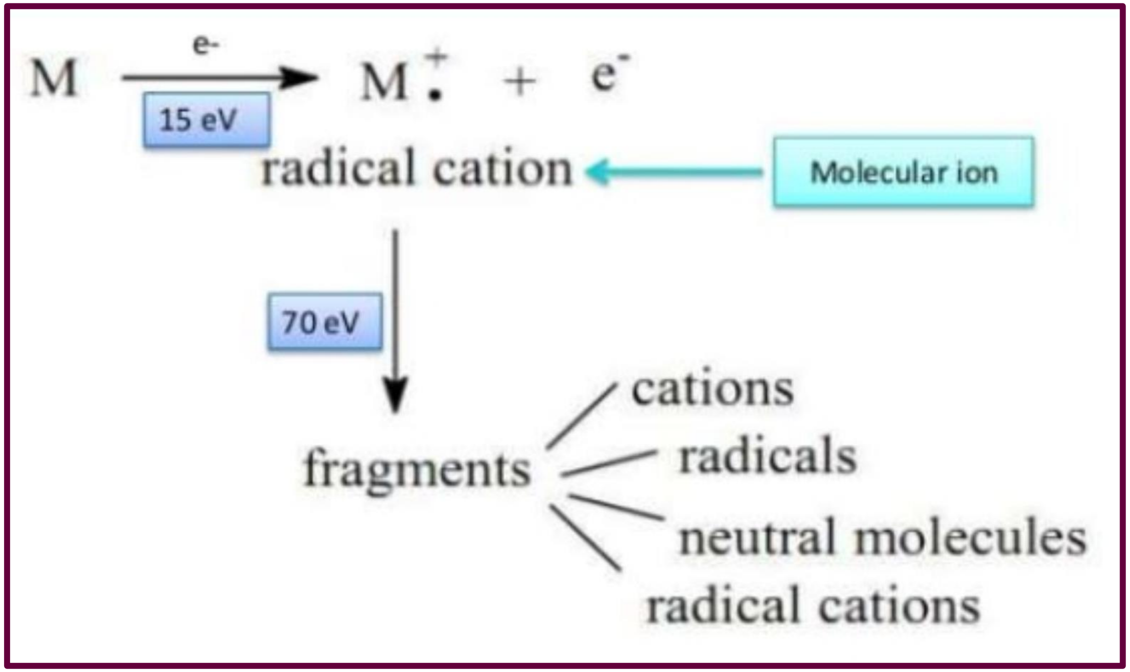
2. The ions are sorted and separated according to their mass and charge. **The Mass Analyzer**

3. The separated ions are then measured, and the results displayed on a chart. **The Detector**

Mass spectrometry : principle

Ejection of an **e**$^-$ and formation of **M**$^{+\bullet}$= radical cation

M is bombarded with a beam of energetic **e**$^-$

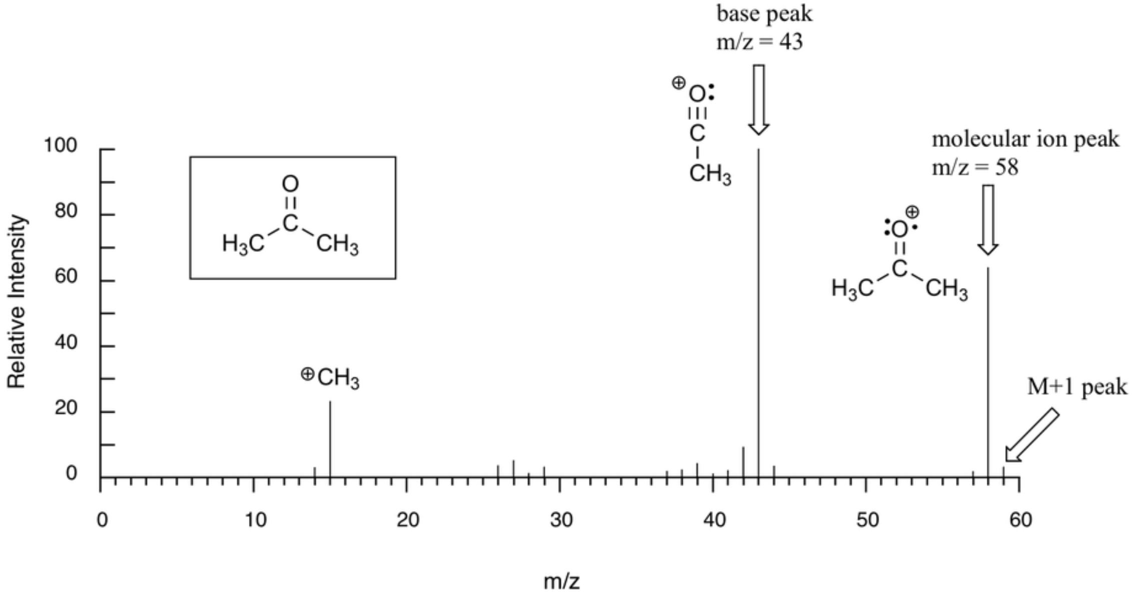


Mass spectrum

= Visualization of the **different types of ions** formed in the source and their **respective intensity** accompanied by their **isotopes**

Key terms for the analysis:

- mass-to-charge ratio (*m/z*)
- relative abundance
- base peak
  (most abundant 100 %)
- molecular ion peak ($M^{+\bullet}$)
- cation radical



Isotopes and MS

→ Table of natural abundance of common elements

		M + 1		M + 2	
	Masse	Masse	%	Masse	%
H	1	2	0,016		
C	12	13	1,08		
N	14	15	0,36		
O	16	17	0,04	18	0,20
F	19				
P	31				
S	32	33	0,78	34	4,39
Cl	35			37	32
Br	79			81	97,5
I	127				

Isotopes and MS

- ✓ It measures individual atoms and enables the isotopic constitution to be determined

✓ **The number of carbons in a molecule:**

carbon is roughly 99% ^{12}C and 1% ^{13}C, then in a molecule containing 10 carbon atoms, the $M^{\bullet +}$ peak will be accompanied by a peak at M+1 having 10% intensity (containing molecules having one ^{13}C atom).

nC = (intensity of M+1/M)*100

✓ **Halogene-containing molecules:**

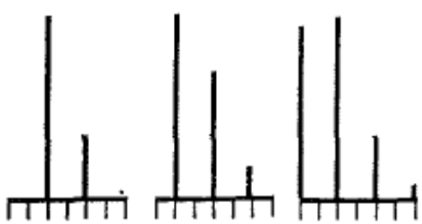


Fig. 6.19 Isotope patterns of Cl, Cl_2, Cl_3 species.

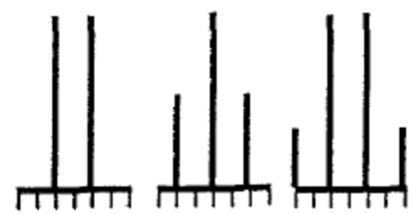


Fig. 6.18 Isotope patterns of Br, Br_2, Br_3 species.

Fragmentations

Note :
it is a cation radical

Ionization of the molecule by Electron Impact (EI)

→ first reaction producing **M+• = molecular ion peak**

$$M + 1e^- \longrightarrow M^{+\bullet} + 2e^-$$

Fragmentations

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Ionization of the molecule by Electron Impact (EI)

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→ first reaction producing **M+$^\bullet$ = molecular ion peak**

Depending on its stability it can fragment :

$M^{+\bullet} \longrightarrow M_1^{+\bullet} + M_2$ giving another **cation radical** + **a neutre**

and/or

$M^{+\bullet} \longrightarrow M_3^{\oplus} + {}^{\bullet}M_4$ giving **a cation** + **a radical**

Fragmentations

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→ the process can continue, depending on the stability of the formed fragments

$M_1^{+\bullet} \longrightarrow M_5^{\oplus} + {}^{\bullet}M_6$

$M_3^{\oplus} \longrightarrow M_7^{\oplus} + M_8$

$M^{+\bullet}$ Ion with an odd number of electron : 1, 3, 5 …
$M^{\oplus}$ Ion with an even number of electron: 0, 2, 4 …

$M_3^{\oplus} \not\longrightarrow M_9^{+\bullet} + {}^{\bullet}M_{10}$ **Impossible fragmentation**

Check that the reaction is in equilibrium

$+\bullet \longrightarrow +\bullet + n$ $\qquad$ $+\bullet \longrightarrow \oplus + \bullet$

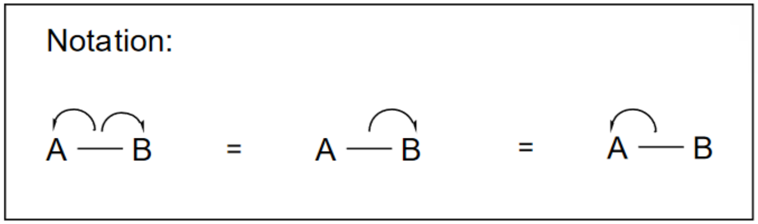
Fragmentations

- **Homolytic cleavage**

$A{\bullet\bullet}B \rightarrow A\bullet + \bullet B$

Single harpoon arrow = 1 e- is moving

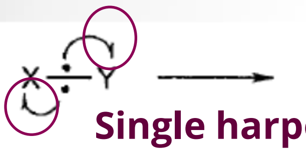
Homolytic cleavage involves movement of single electrons.



Fragmentations

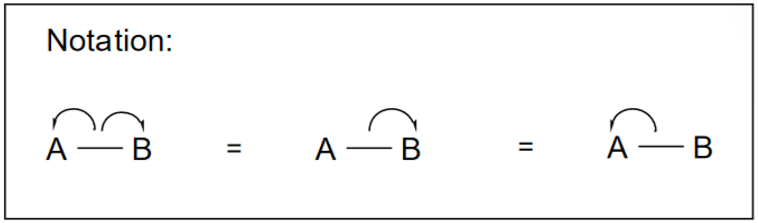
- **Homolytic cleavage**

$A{\bullet\bullet}B \rightarrow A\bullet + \bullet B$



Single harpoon arrow = 1 e- is moving

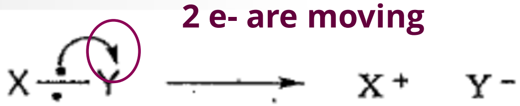
Homolytic cleavage involves movement of single electrons.



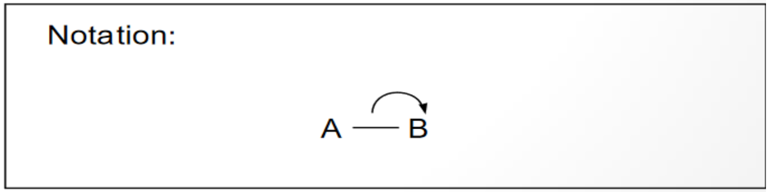
- **Heterolytic cleavage**

$A{\bullet\bullet}B \rightarrow A^{+} + {\bullet\bullet}B^{-}$

Note : double harpoon arrow 2 e- are moving



Heterolytic bond cleavage involves movement of a pair of electrons.



- Simple fragmentations (1 bond is cleaved)

- Transpositions (2 bonds are cleaved)

MS : the "nitrogen rule"

The nitrogen rule states that organic compounds containing exclusively hydrogen, carbon, nitrogen, oxygen, silicon, phosphorus, sulfur, and the halogens having :

an **even molecular weight** contains
an even number of nitrogen (0, 2, 4 …)

an **odd molecular weight** contains
an odd number of nitrogen (1, 3, 5 …)

→ The nitrogen rule is a general principle very useful when attempting to solve organic mass spectrometry structures

MS : the “nitrogen rule”

	Even Mass (0, 2, 4…)	Odd Mass (1, 3, 5…)
$R^{+\bullet}$	even number of N	odd number of N
R^{+}	odd number of N	even number of N

In practice, in a molecule **without nitrogen**:

- An ion having an even mass is a cation radical $M^{+\bullet}$
- An ion having an odd mass is a cation M^{+}

MS rules : simple fragmentations and transpositions

In practice, in a molecule **without a nitrogen** (**$M^{+\bullet}$ is even 2, 4, 6…**)

▪A **simple fragmentation** generates
a charged fragment and a neutral radical, both having an odd mass

$$\underset{\text{Even mass}}{M^{+\bullet}} \longrightarrow \underset{\text{Odd mass}}{M_1^{+}} + \underset{\text{Odd mass}}{M_2^{\bullet}} \quad \text{(cation + radical)}$$

▪ A **transposition** generates
a cation radical and a neutral molecule

$$\underset{\text{Even mass}}{M^{+\bullet}} \longrightarrow \underset{\text{Even mass}}{M_3^{+\bullet}} + \underset{\text{Even mass}}{M_4} \quad \text{(cation radical + neutral)}$$

Degree of unsaturation

$$\text{Degree of unsaturation} = \frac{2C + 2 + N - H - X}{2}$$

Exercises

Let's practice with Electron Impact exercises